PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	MacNeil et al.		
Serial No.:	10/520,566	Case No.: 21141YP	Art Unit: 1614
Filed:	January 7, 2005		Examiner: Kevin E. Weddington
For:	COMBINATION THERAPY FOR THE TREATMENT OF OBESITY		

Mail Stop AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

I, Dr. Douglas John MacNeil hereby declare:

1. I am currently employed by Merck & Co., Inc. as a Director of Metabolic Disorders, and have been employed as a scientist at Merck & Co., Inc. since 1979. Part of my responsibilities at Merck Research Laboratories at Merck & Co., Inc. include work on projects involving the pharmacology of obesity and metabolic disorders, including interpretation of *in vivo* pharmacology research in testing compounds for their use to treat obesity and metabolic syndrome.

2. My educational background is as follows:

School	Date	Major	Degree
Massachusetts Institute of Technology	1970-74	Life Science	B.S.
Massachusetts Institute of Technology	1970-74	Chemistry	B.S.

University of Wisconsin, Madison	1975-77	Bacteriology	M.S.
University of Wisconsin, Madison	1977-79	Bacteriology	Ph.D.

Under my thesis advisor Professor Winston Brill, I studied the genetics and biochemistry of nitrogen fixation. I then completed a 2 year post doctoral study with Dr. Bonnie Tyler at Merck Research Labs in which I studied the role of nitrogen regulation in metabolism.

3. My academic experience is as follows:

1974 Undergraduate Research Assistant, Massachusetts Institute of Technology
1976 Graduate Teaching Assistant, University of Wisconsin
2004-2007 Thesis advisor Lydia Kuo, Georgetown University

4. My employment experience at Merck & Co., Inc. is as follows:

Title	From	To
Director	2003	Present
Senior Investigator	2000	2003
Senior Research Fellow	1994	2000
Research Fellow	1988	1994
Senior Research Microbiologist	1981	1988
Postdoctoral Scientist	1979	1981

5. My honors and awards are as follows:

1970-1974	Firestone/National Merit Scholar
1975-1977	Wisconsin Alumnae Foundation Fellowship
1977-1979	NIH Predoctoral Trainee, University of Wisconsin
2002	Contributor to ASM Careers Symposium
1982-2002	Reviewer for: Gene, J. of Bacteriology, Molec. and Cell. Biology, Br. J. of Pharmacol.
2004	Rahway Research Recognition Award: Mc4r Back-up PCC
2004	Rahway Research Recognition Award: MCH1R 1st PCC

2005	Rahway Research Recognition Award: License PYY3-36
2006	Organizing Committee 8Th International NPY Conference, Tampa, FL
2006	Symposium co-chair 8Th International NPY Conference, Tampa, FL
2005-2006	Reviewer for Eu. J. of Pharmacology, Physiology and Behavior
2006	Rahway Research Recognition Award: in vivo Validation Group
2006	Rahway Research Recognition Award: Viral Validation Group
2006	Rahway Research Recognition Award: MCH1R 2nd PCC
2007	Reviewer for Cur. Top, in Med. Chem., BBSRC

6. My society memberships are as follows:

Society for Neuroscience
Biochemical Pharmacology Discussion Group, New York Academy of Sciences
North American Association for the Study of Obesity

7. My presentations are as follows:

1979. Genetic manipulation of nitrogen fixation genes. (Symposium on genetic manipulation of photosynthetic and nitrogen fixing microbes). 7th Annual Mtg. of American Society of Photobiology.

1980. Genetics and regulation of nitrogen fixation genes in *Klebsiella pneumoniae*. (Symposium on Molecular Genetics of Nitrogen Fixation). Annual Mtg. of American Society for Microbiology.

1985. Cloning vectors for *Streptomyces avermitilis*. University of Wisconsin, Department of Pharmacy Seminar.

1985. Derivatives of pVE1 and their introduction into *S. avermitilis*. 29th Wind River Conference on Genetic Exchange.

1986. Efficient transformation of *Streptomyces avermitilis*. 43rd Annual Mtg. of Society of Industrial Microbiology.

1987. Plasmids of *Streptomyces*. (Symposium on New Horizons in Actinomycete Biology). 87th Annual Mtg. of American Society for Microbiology.

1988. Characterization of a unique methyl-specific restriction system in *Streptomyces avermitilis*. 45th Annual Mtg. of the Society of Industrial Microbiology.

1989. Cloning genes for avermectin biosynthesis. Guest lecturer, Bacteriology 875: "Secondary metabolism in microorganisms: Biotechnology of drug production". University of Wisconsin.

1989. Cloning genes for avermectin biosynthesis. University of Connecticut.

1990. The genes for avermectin biosynthesis are clustered. UCLA Symposium on Molecular Biology of Streptomycetes.

1990. Transposon mutagenesis and gene cluster displacement. UCLA Symposium on Molecular Biology of Streptomycetes.

1991. Cloning the Genes for Avermectin Biosynthesis. Ohio State University

1991. Expression of the Genes for Avermectin Biosynthesis. (Symposium on Gene Expression in Secondary Metabolism) 1991 ASM Conference on Biotechnology

1991. Complex Organization of the Avermectin Polyketide Synthase genes. (Focal Point Discussion 4: Polyketide Synthases) Eighth International Symposium on Biology of Actinomycetes

1991. Co-convenor (Biochemistry and Regulation of Secondary Metabolites) Eighth International Symposium on Biology of Actinomycetes

1992. Biotechnology at Merck. Clarion University

1992. A comparison of the genes encoding the polyketide synthases for avermectin and nemadectin. (Symposium on Genetics of Secondary Metabolite Biosynthesis II) Fifth ASM Conference on Genetics and Molecular Biology of Industrial Microorganisms

1992. Modular organization of the genes for synthesis of the polyketide in the antiparasitic compound avermectin. (Department of Bacteriology Seminar) University of Wisconsin

1993. Correlation of the avermectin polyketide synthase genes to the avermectin structure: Implications for designing novel avermectins (Symposium on Secondary Metabolites) Recombinant DNA Technology II, Engineering Foundation Conference

1993. Modular organization of the genes encoding the polyketide synthases involved in biosynthesis of the antiparasitic compounds avermectin and nemadectin. (Department of Chemistry Seminar) Brown University

1994. Molecular Biology of Avermectins. Joint Japan-US NSF Conference on Streptomycetes. Invited Speaker.

2000. A Pharmacological Characterization of the Murine NPY Y1, Y2, Y4, Y5, and y6 Receptors. Winter Neuropeptide Conference.

2000. Characterization of Selective Ligands for the Murine NPY Receptors. 13th International Symposium on Regulatory Peptides, Cairns, Australia Oct. 22-26, 2000

2000. Characterization of several NPY ligands with cloned murine NPY receptors: ICV administration of a selective Y5 agonist, D-Trp^{34}NPY, induces obesity. 11th International Congress of Endocrinology, Sydney Australia, Oct. 30 –Nov. 2, 2000.

2004: Discovering New Anti-Obesity Therapeutics. Georgetown University. Nov 15, 2004

2005: Potent, selective novel NPY Y5 antagonists. North American Association For the Study Of Obesity Annual Scientific 2005 Meeting (NAASO), Vancouver, British Columbia, 10/15/05 - 10/19/05

2006. A Y5 antagonist causes weight loss in diet-induced obese rhesus monkeys. For presentation at: Keystone Symposium: Gut Hormones and Other Regulators of Appetite, Satiety and Energy Expenditure Santa Fe, NM 3/2/06-3/7/06

2006: NPY5R, MC4R and CB1R: How important is each in modulating obesity? Invited Symposium Presentation at the 8Th International NPY Meeting, St. Petersburg, FL, Apr 22-26, 2006.

8. My publications are as follows:

1. Fischhoff, D., MacNeil, D., and Kleckner, N. (1976) Terminal redundancy heterozygotes involving the first-step-transfer region of the bacteriophage T5 chromosome. Genetics 82: 145-159.

2. MacNeil, D., and Brill, W.J. (1978) 6-Cyanopurine, a color indicator useful for isolating mutations in the nif (nitrogen fixation) genes of Klebsiella pneumoniae. J. Bacteriol. 136: 247-252.

3. MacNeil, D., MacNeil, T., and Brill, W.J. (1978) Genetic modifications of N2-fixing systems. Bioscience 28: 576-578.

4. MacNeil, T., MacNeil, D., Roberts, G.P., Supiano, M.A., and Brill, W.J. (1978) Fine structure mapping and complementation analysis of nif (nitrogen fixation) genes in Klebsiella pneumoniae. J. Bacteriol. 136: 253-266.

5. Roberts, G.P., MacNeil, T., MacNeil, D., and Brill, W.J. (1978) Regulation and characterization of protein products coded by the nif (nitrogen fixation) genes of Klebsiella pneumoniae. J. Bacteriol. 136: 267-279.

6. MacNeil, D., Supiano, M.A., and Brill, W.J. (1979) Order of genes near nif in Klebsiella pneumoniae. J. Bacteriol. 138: 1041-1045.

7. MacNeil, D., Howe, M.M., and Brill, W.J. (1980) Isolation and characterization of lambda specialized transducing bacteriophages carrying Klebsiella pneumoniae nif genes. J. Bacteriol. 141: 1264-1271.

8. MacNeil, T., Roberts, G.P., MacNeil, D., Supiano, M.A., and Brill, W.J. (1980) Regulation and genetics of nitrogen fixation in Klebsiella pneumoniae. Nitrogen Fixation, Vol. 1, 63-70. (W.E. Newton and W.H. Orme-Johnson, eds.) University Park Press, Baltimore.

9. MacNeil, D., and Brill, W.J. (1980) Mutations in nif genes that cause Klebsiella pneumoniae to be derepressed for nitrogenase synthesis in the presence of ammonium. J. Bacteriol. 144: 744-751.

10. MacNeil, D., Zu, J., and Brill, W.J. (1981) Regulation of nitrogen fixation in Klebsiella pneumoniae: isolation and characterization of strains with nif-lac fusions. J. Bacteriol. 145: 348-357.

11. MacNeil, D. (1981) Genetics and Regulation of nitrogen fixation in Klebsiella pneumoniae. Microbiology, 1981, 81-84. (D. Schlessinger, ed.). American Society for Microbiology, Washington, D.C.

12. MacNeil, D. (1981) A general method using Mu-Mudl dilysogens to determine the direction of transcription and generate deletions in the glnA region of Escherichia coli. J. Bacteriol. 146: 260-268.

13. MacNeil, T., MacNeil, D., and Tyler, B. (1982) Fine-structure deletion map and complementation analysis of the glnA-glnL-glnG region in Escherichia coli. J. Bacteriol. 15: 1302-1313.

14. MacNeil, T., Roberts, G.P., MacNeil, D., and Tyler, B. (1982) The products of glnL and glnG are bifunctional regulatory proteins. Mol. Gen. Genet. 188: 325-333.

15. Jayakumar, A., Schulman, I., MacNeil, D., and Barnes, E.M. Jr. (1986) Role of the Escherichia coli glnALG operon in regulation of ammonium transport. J. Bacteriol. 166: 281-284.

16. MacNeil, D.J. (1986) A flexible boiling procedure for isolating plasmid DNA from Gram-positive microorganisms. J. Microbiol. Methods 5: 115-123.

17. MacNeil, D.J. and Klapko, L.M. (1986) Transformation of plasmid DNA into Streptomyces avermitilis. J. of Industrial Microbiol. 2: 209-218.

18. MacNeil, D.J. (1987) Introduction of plasmid DNA into Streptomyces lividans by electroporation. FEMS Microbiology Letters 42: 239-244.

19. MacNeil, D.J. (1988) Characterization of a unique methyl-specific restriction system in Streptomyces avermitilis. J. Bacteriol. 170: 5607-5612.

20. Streicher, S.L., Ruby, C.L., Paress, P.S., Sweasy, J.B., Danis, S.J., MacNeil, D.J., Gewain, K., MacNeil, T., Foor, F., Morin, N., Cimis, G., Rubin, R., Goldberg, R., Nallin, M., Schulman, M.D., and Gibbons, P. (1989) Cloning genes for avermectin biosynthesis in Streptomyces avermitilis. In Genetics and Molecular Biology of Industrial Microorganisms, p44-59. (C.L. Hershberger, S.W. Queener, and G. Hegeman, eds.) American Society for Microbiology, Washington, D.C.

21. MacNeil, D.J., Gewain, K.M., Ruby, C.L. Dezeny, G., Gibbons, P.H. and T. MacNeil (1992) Analysis of the Streptomyces avermitilis avermectin genes using a novel integration vector. Gene 111: 61-68.

22. Berg, C.M., Vartak, N.B., Wang, G., Xu, X.X., Liu, L., MacNeil, D.J., Gewain, K.M., Wiater, L. A. and Berg, D. E. (1992) mγδ-1, a small γδ (Tn1000) derivative useful for plasmid mutagenesis, allele replacement and DNA sequencing. Gene 113: 9-16.

23. MacNeil, D.J., Occi, J L., Gewain, K.M., MacNeil, T., Gibbons, P., Ruby, C L., and Danis, S.J. (1992) Complex organization of the Streptomyces avermitilis genes encoding the avermectin polyketide synthase. Gene 115: 119-125.

24. Gewain, K.M., Occi, J.L., Foor, F., and MacNeil, D.J. (1992) Vectors for generating nested deletions and to facilitate subcloning G+C-rich DNA between Escherichia coli and Streptomyces sp. Gene 119: 149-150.

25. MacNeil, T., Gewain, K.M., and MacNeil, D.J. (1993) Deletion analysis of the avermectin biosynthetic genes of Streptomyces avermitilis by Gene Cluster Displacement. J. Bacteriol. 175: 2552-2563.

26. Occi, J.L., Gibbons, P.H., Wong, E., and MacNeil, D.J. (1993). Insertion of transposon Tn5seq1 into G+C-rich DNA of Streptomyces avermitilis: generation of 8-, 9- and 10-bp duplications. Plasmid 30: 167-169.

27. MacNeil, D. J., Occi, J. L., Gewain, K.M., MacNeil, T., Gibbons, P.H., Foor, F., and Morin, N. (1993) A comparison of the genes encoding the polyketide synthases for avermectin, erythromycin, and nemadectin. In Industrial Microorganisms: Basic and Applied Molecular Genetics, p245-256. (R. H. Baltz, G. D. Hegeman, and P. L. Skatrud, eds.) American Society for Microbiology, Washington, D.C.

28. MacNeil, D. J., Occi, J.L., Gewain, K.M., MacNeil, T. (1994) Correlation of the avermectin polyketide synthase genes to the avermectin structure: implications for designing novel avermectins. Annals N. Y. Acad. Sci. 721: 123-132.

29. MacNeil, D.J., Occi, J.L., Hey, P.J., Strader, C.D., and Graziano, M.P. (1994) Cloning and expression of a human glucagon receptor. Biochem. Biophys. Res. Commun. 198: 328-334.

30. MacNeil, D. (1995) Avermectins. In Genetics and Biochemistry of Antibiotic Production, p421-442. (L.C. Vining and C. Stuttard, eds.) Butterworths, Boston.

31. Weinberg, D.H., Sirinathsinghji, D.J.S., Tan, C.P., Shiao, L., Morin, N., Rigby, M.R., Heavens, R.H., Rapoport, D.R., Bayne, M.L., Cascieri, M.A., Strader, C.D., Linemeyer, D.L., and MacNeil, D.J. (1996) Cloning and expression of a novel neuropeptide Y receptor. J. Biolog. Chem. 271: 16435-16438.

32. Madduri, K., Kennedy, J. Rivola, G., Inventi-Solari, A., Zanuso, G., Colombo, A. L., Gewain, K.M., Occi, J.L., MacNeil, D.J., and Hutchinson C.R. (1998) Production of the antitumor drug Epirubicin (4'-epidoxorubicin) and its precursor, 4'-epidaunorubicin, by a genetically engineered strain of Streptomyces peucetius. Nature Biotechnology. 16: 69-75.

33. Kanatani, A., Ito, J., Ishihara, A., Iwaasa, H., Fukuroda, T., Fukami, T., MacNeil, D.J., Van der Ploeg, L.H.T., and Ihara, M. (1998) NPY-induced feeding involves the action of a Y1-like receptor in rodents. Regulatory Peptides 75:409-415.

34. Trivedi, P., Yu, H., MacNeil, D.J., Van der Ploeg, L. H., and Guan, X-M. (1998) Distribution of Orexin receptor mRNA in the rat brain. FEBS Lett. 438:71-75.

35. Tan, C.P., McKee, K.M., Weinberg, D.H., MacNeil, T., Palyha, O.C., Feighner, S.D., Hreniuk, D.L., Van der Ploeg, L.H.T., MacNeil, D.J., and Howard, A.D. (1999) Molecular analysis of a new splice variant of the human melanocortin-1 receptor. FEBS Lett. 451:137-141.

36. Feighner, S.D., Tan, C.P., McKee, K.K., Palyha, O.C., Hreniuk, D. L., Pong, S.S., Austin, C.P., Figueroa, D., MacNeil, D., Cascieri, M.A., Nargund, R., Bakshi, R., Abramovitz, M., Stocco, R., Kargman, S., O'Neill, G., Van der Ploeg, L.H.T., Evans, J., Patchett, A.A., Smith, R.G., and Howard, A.D. (1999) Receptor for Motilin Identified in the Human Gastrointestinal System. Science 284: 2184-2188.

37. Kanatani, A., Mashiko, S., Murai, N., Sugimoto, N., Ito, J., Fukuroda T., Fukami T., MacNeil, D.J., Van der Ploeg, L.H.T., Saga Y., and Ihara, M. (2000) Central Role of the Y1 receptor in NPY-mediated Feeding Regulation: Comparative study in wild type, Y1 receptor deficient and Y5 receptor deficient mice. Endocrinology 141: 1011-1016.

38. MacNeil, D. J. and Weinberg, D.H. (2000) Homology based cloning methods: identification of the NPY Y2, Y4, and Y6 receptors. In Methods in Molecular Biology: Neuropeptide Y Protocols. (A. Balasubramaniam, ed.) Humana Press, Totawa.

39. MacNeil, D.J., Morin, N.R., Beck-Sickinger, A.G., Kanatani, A., Asahi, S., Ishihara, A., Ihara, M., and van der Ploeg, L.H.T. (2000) A Pharmacological Characterization of the Murine NPY Y1, Y2, Y4, Y5, and y6 Receptors. Regulatory Peptides 86:69.

40. Kanatani, A., Ishihara, A., Iwaasa, H., Nakamura, K., Okamoto, O., Hidaka, M., Ito, J., Fukuroda, T., MacNeil, D.J., Van der Ploeg, L.H.T., Ishii, Y., Okabe, T., Fukami T., and Ihara, M. L-152,804: Orally-active and Selective Neuropeptide Y5 antagonist. (2000) Biochem. Biophys. Res. Commun. 272:169-173.

41. Sailer, A., Sano, H., Zeng, Z., McDonald, T.P., Pan, J., Pong, S.-S., Feighner, S.D., Tan, C.P., Fukami, T., Iwaasa, H., Hreniuk, D.L., Morin, N., Sadowski, S., Nossoughi, R., Ito, M., Ito, M., Bansal, A., Ky, B., Figueroa, D.J., Jiang, Q., Austin, C.P., MacNeil, D.J., Ishihara, A., Ihara, M., Kanatani, A., Van der Ploeg, L.H.T., Howard, A.,D., and Liu, Q. Identification and characterization of a second melanin-concentrating hormone receptor, MCH-2R. (2001) PNAS, 98:7564-7569.

42. Wohlert, S-E., Lomovskaya, N., Kulowski, K., Fonstein, L., Occi, J.L., Gewain, K.M., MacNeil D.J., and Hutchinson, C.R. Insights about the biosynthesis of the avermectin deoxysugar L-oleandrose through heterologous expression of Streptomyces avermitilis deoxysugar genes in Streptomyces lividans. (2001) Chemistry and Biology, 8: 681-700

43. Bednarek, M.A., Feighner, S.D., Hreniuk, D.L., Palyha, O.C., Morin, N.R., Sadowski, S.S., MacNeil, D.J., Howard, A.D., and van der Ploeg, L.H.T. Short segment of human melanin-concentrating hormone (hMCH) is sufficient for full activation of human melanin-concentrating hormone receptors 1 and 2. (2001) Biochemistry, 40:9379-9386.

44. Smith, C.J., Morin, N.R., Bills, G.F., Dombrowski, A.W., Salituro, G.M, Smith, S.K., MacNeil, Zhao, A., and MacNeil, D.J. Novel Sesquiterpenoids from the Fermentation of Xylaria persicaria are Selective Ligands for the NPY Y5 Receptor. (2002) J. Org. Chem., 67:5001-5004.

45. Marsh, D.J., Weingarth, D.T., Novi, D.E., Chen, H.Y., Trumbauer, M.E., Chen, A.S., Frazier, E.G., Shen, Z., Feng, Y., Guan, X., Jiang, M.M., Yu, H., Metzger, J.M., Kuca, S.J., Shearman, L.P., Camacho, R.E., Gopal-Truter, S., MacNeil, D.J., MacIntyre, D.E., Van der Ploeg, L.H.T., and Qian, S. Melanin-concentrating hormone 1 receptor-deficient mice are lean, hyperactive, hyperphagic, and have altered metabolism. (2002) PNAS, 99:3240-3245.

46. Qian, S., Chen, H., Weingarth, D., Trumbauer, M.E., Novi, D., Guan, X., Yu, H., Zhu, S., Feng, Y., Frazier, E., Chen, A., Metzger J.M., Kuca, S.J., Camacho, R.E., Shearman, L.P., Gopal-Truter, S., MacNeil, D.J., Van der Ploeg, L.H.T., and Marsh, D.J. Neither AgRP nor NPY are critically required for the regulation of energy homeostasis in mice. (2002) Molecular and Cellular Biology, 22:5027-5035.

47. MacNeil, D.J., Howard, A. D., Guan, X ., Fong, T., Nargund, R.P., Bednarek, M.A., Goulet, M.T., Weinberg, D.H., Strack, A.M., Marsh, D.J., Chen, H.Y., Shen, C.-P., Chen, A.S., Rosenblum, C.I., MacNeil, T., Tota, M.R., and Van der Ploeg, L.H.T. The role of Melanocortins in body weight regulation: opportunities for the treatment of obesity. (2002) E. J. Pharmacol. 450:93-109..

48. Bednarek, M.A., Hreniuk, D.L., Tan, C., Palyha, O.C., MacNeil, D.J., Van der Ploeg, L.H.Y., Howard, A.D., and Feighner, S.D. Synthesis and Biological Evaluation in Vitro of Selective, High Affinity Peptide Antagonists of Human Melanin-Concentrating Hormone Action at Human Melanin-Concentrating Hormone Receptor 1. (2002) Biochemistry, 41:6383-6390.

49. Bednarek, M.A., Tan, C., Hreniuk, D.L., Palyha, O.C., MacNeil, D.J., Van der Ploeg, L.H.Y., Howard, A.D., and Feighner, S.D. Synthesis and biological evaluation in vitro of selective, high potency peptide agonists of human melanin-concentrating hormone action at human melanin-concentrating hormone receptor 1. (2002) J. Biol. Chem., 277: 13821-13826.

50. Tan, C.P., Sano, H., Iwassa, H., Pan, J., Sailer, A.W., Hreniuk, D.L., Feighner, S.D., Palyha, O.C., Figuero, D.J., Austin, C.P., Jiang, M.M., Yu, H., Ito, J., Ito, M., Ito, M., Guan, X-M., MacNeil, D.J., Kanatani, A., Van der Ploeg, L.H.T., Howard, A. D. Melanin-concentrating hormone receptor subtypes 1 and 2: Species-specific gene expression. (2002) Genomics, 79:785-792.

51. Mashiko, S., Ishihara, A., Iwaasa, H., Sano, H., Oda, Z., Ito, J., Yumoto, M., Okawa, M., Suzuki, J., Fukuroda, T., Jitsuoka, M., Morin, N.R., MacNeil, D.J., Van der Ploeg, L.H.T., Fukami, T., and Kanatani, A. Characterization of NPY Y5 receptor-mediated obesity in mice: Chronic intracerebroventricular infusion of D-Trp34NPY. (2003) Endocrinology 144: 1793-1801.

52. Chung, C., Varnerin, J., Morin, N.R., MacNeil, D.J., Singh, S.B., Patel, S., Scapin, G., van der Ploeg, L.H.T., Tota, M.R. The role of tryptophan 1072 in PDE3B inhibitor binding. (2003) Biochemical and Biophysical Research Communications. 307: 1045-50.

53. Varnerin, J.P., Chung, C.C., Patel, S.B., Scapin, G., Parmee, E.R., Morin, N.R, MacNeil, D.J., Cully, D.F., Van der Ploeg, L.H.T., and Tota, M.R. Expression, Refolding and Purification of Recombinant Human Phosphodiesterase 3B: Definition of the N-Terminus of the Catalytic Core. (2004) Protein Expression and Purification. Protein Expression and Purification. 35: 225-236.

54. Chen, H.Y., Trumbauer, M.E., Chen, A.S., Weingarth, D.T., Frazier, E.G., Shen, Z., Ye, Z., Nargund, R.P., Feighner, S.D., Marsh, D.J., Guan, X., Van der Ploeg, L.H.T., Howard, A.D., MacNeil, D.J., Qian, S. (2004) Orexigenic Action of Peripheral Ghrelin is Mediated by Neuropeptide Y (NPY) and Agouti-Related Protein (AgRP). Endocrinol. 145:2607-2612.

55. Chen, R.Z., Huang, R.R. C., Shen, C.-P., MacNeil, D.J., Fong, T.M. (2004) Synergistic effects of cannabinoid inverse agonist AM251 and opioid antagonist nalmefene on food intake in mice. Brain Research. 999:227-230.

56. Chen, R.Z., Huang, R.-R.C., Shen, C.-P., MacNeil, D.J., Fong, T.M. (2004) Chronic administration of nalmefene leads to increased food intake and body weight gain in mice. Eu. J. Pharmacol. 495:63-66.

57. Hayes, D.M., Knapp, D.J., MacNeil, D.J. and Thiele, T.E. (2004) Source Peripheral and central administration of a selective neuropeptide YY1 receptor antagonist suppresses ethanol intake by C57BL/6J mice. Alcoholism Clinical and Experimental Research 28: 1324-1330.

58. Unmehopa, U.A., van Heerikhuize, J.J., Spijkstra, W., Woods,J.W., Howard, A., Zycband, E., Feighner, S., Hreniuk, D., Palyha, O., Guan, X.-M., MacNeil, D.J., Van der Ploeg, L.H.T., and Swaab, R.F. (2005) Increased Melanin Concentrating Hormone Receptor Type I (MCH1R) in the Human Hypothalamic Infundibular Nucleus in Cachexia. J. Clin. Endocrinol and Metab. 90: 2412-2419.

59. Mashiko, S., Ishihara, A., Gomori, A., Moriya, R., Ito, M., Iwaasa, H., Matsuda, M., Marsh, D.J., Feng, Y., Bednarek, M.A., MacNeil, D.J., and Kanatani A. (2005) Anti-obesity Effect of an MCH-1R Antagonist in Diet-Induced Obese Mice, Endo., 146:3080-3086.

60. Chen, A.S., Mumick, S., Zhang, C., Lamb, J.R., DAI, H., Weingarth, D.T., Chen, H.Y., MacNeil, D.J., Reitman, M.L., Qian, S. (2005) Diet Induction of monocyte chemoattractant protein-1 and its impact on obesity. Obesity Research 13:1311-1320.

61. MacNeil, D.J. and Kanatani, A. (2005) NPY and Energy Homeostasis: Opportunities for Novel Anti-Obesity Therapies, in NPY Family of Peptides in Neurobiology, Cardiovascular and Metabolic Disorders: from Genes to Therapeutics, eds. Zukowska, Z. and. Feuerstein, G. Birkhauser Verlag, Basel

62. Ito, S., Yoshimoto, R., Miyamoto, Y., Mitobe, Y., Nakamura, T., Ishihara, A., MacNeil, D.J., Kanatani A., and Tokita, S. (2006) Detailed pharmacological characterization of GT-2331 for the rat histamine H3 receptor. Eur. J. Pharmacol. 529: 40-46.

63. Ishihara, A. , Kanatani, A., Mashiko, S., Tanaka, T., Hidaka, M., Gomori, A., IWAASA, H., Murai, N., Egashira, S., Mitobe, Y., Matsushita, H., Okamoto, O., Sato, N., Jitsuoka, M., Fukuroda, T., Ohe, T., Guan, X., MacNeil, D.J., Van Der Ploeg, L.H., Nishikibe, M., Ishii, Y., Ihara, M., Fukami, T. (2006) A neuropeptide Y Y5 antagonist selectively ameliorates body weight gain and associated parameters in diet-induced obese mice. Proc Natl Acad Sci U S A. 103:7154-8.

64. Kanatani, A., Ishihara, A., Iwaasa, H., MacNeil, D.J., Fukami, T. (2006) Development of NPY antagonists and their anorectic effects. Nippon Yakurigaku Zasshi. (Folia Pharmacologica Japonica) 127:88-91.

65. Yoshimoto, R. , Miyamoto, Y., Shimamura, K., Ishihara, A., Takahashi, K., Kotani, H., Chen, A.S., Chen, H.Y., MacNeil, D.J., Kanatani, A., Tokita, S. (2006) Therapeutic potential of histamine H3 receptor agonist for the treatment of obesity and diabetes mellitus. Proc Natl Acad Sci U S A 103: 13866-13871.

66. Van Der Ploeg, L.H.T., Kanatani, A., MacNeil, D.J., Fong, T.M., Strack, A.M., Nargund, R.P., Guan, X. (2006) Design and synthesis of (ant)-agonists that alter appetite and adiposity. Progress in Brain Research. 153: 107-118.

67. Erondu, N.E., Gantz, I., Musser, B.J., Suryawanshi, S., Mallick, M., Addy, C., Cote, J., Bray, G.A., Fujioka, K., Bays, H.E., Hollander, P., Sanabria, S.M., Eng, W., Langstrom, B., Hargreaves, R.J., Burns, H.D., MacNeil, D.J., Gottesdiener, K.M., Amatruda, J., Kaufman, K.D., Heymsfield, S.B. (2006) Neuropeptide Y5 receptor antagonism does not induce clinically meaningful weight loss in overweight and obese adults. Cell Metabolism. 4: 275-282.

68. Jiang, J., Lin, P., Hoang, M., Chang, L., Tan, C.P., Feighner, S.D., Palyha, O.C., Hreniuk, D.L., Pan, J., Sailer, A.W., Morin, N.R., MacNeil, D.J., Howard, A.D., Van Der Ploeg, L.H.T., Goulet, M.T., Devita, R.J. (2006) 4-aminoquinoline melanin-concentrating hormone 1-receptor (MCH1r) antagonists. Bioorganic and Medicinal Chemistry Letters 20:5275-5279.

69. Jiang, J., Hoang, M., Young, J.R., Chaung, D., Eid, R., Turner, C.L., Lin, P., Tong, X.S., Wang, J., Tan, C.P., Feighner, S.D., Palyha, O.C., Hreniuk, D.L., Pan, J., Sailer, A.W., MacNeil, D.J., Howard, A.D., Shearman, L.P., Stribling, D.S., Camacho, R.E., Strack, A.M., Van Der Ploeg, L.H.T., Goulet, M.T., Devita, R.J. (2006) 2-aminoquinoline melanin-concentrating hormone (MCH)1r antagonists. Bioorganic & Medicinal Chemistry Letters 20:5270-5274.

70. Ishihara, A., Moriya, M., MacNeil, D.J., Fukami, T., Kanatani, A. (2006) Neuropeptide Y receptors as targets of obesity treatment. Expert Opinion on Therapeutic Patents 16:1701-1712.

71. Mashiko, S, Ishihara, A., Iwaasa, H., Sano, H., Ito, J., Gomori, A., Oda, Z., Moriya, R., Matsushita, H., Jitsuoka, M., Okamoto, O., MacNeil, D.J., Van Der Ploeg, L.H.T., Fukami, T., Kanatani, A. (2007) A pair-feeding study reveals that a Y5 antagonist causes weight loss in diet-induced obese mice by modulating food intake and energy expenditure. Mol. Pharmacol. 71: 602-608.

72. Gantz, I., Erondu, N.E., Mallick, M., Musser, B.J., Krishna, R., Tanaka, W.K., Snyder, K.M., Stevens, C., Stroh, M.A., Zhu, H., Wagner, J.A., MacNeil, D.J., Heymsfield, S.B., Amatruda, J. (2007) Efficacy and safety of intranasal peptide YY3-36 for weight reduction in obese adults. J. Clin. Endocrinol. & Metab. 92: 1754-1757.

73. Gomori, A., Ishihara, A., Ito, M., Matsushita, H., Ito, M., Mashiko, S., Iwaasa, H., Matsuda, M., Bednarek, M.A., Qian, S., MacNeil, D.J., Kanatani, A. (2007) Blockade of MCH1 receptor signaling ameliorates obesity and related hepatic steatosis in ovariectomized mice. Brit. J. Pharmacol. 151: 900-908.

9. I am familiar with Claims 56-60 and 73 of Merck's pending patent application 10/520,566. I have discussed this patent application and Claims 56-60 and 73 of this patent application with the patent attorney of record.

10. The *in vivo* studies performed on the combination of NPY5 antagonist L-753550 (3-oxo-N-(5-phenyl-2-pyrazinyl)-spiro[isobenzofuran-1(3H),4'-piperidine]-1'-carboxamide, compound 1 of Claim 58 of the present application) and NPY1 receptor antagonist J-115,814 ((L-753478, ([(-)-2-[1-(3-chloro-5-isopropyloxycarbonylaminophenyl) ethylamino]-6-[2-(-ethyl-4-methyl-1,3-thiazol-2-yl)ethyl]-4morpholinopyridine])) described below, and for which results are described in attached Exhibits 1 and 2, were conducted under my direction. Protocols for the studies and results are discussed herein.

11. Exhibits 1 and 2 are graphs showing the effect of the combination of a NPY5 receptor antagonist L-753550 (30 mg/kg po) and NPY1 receptor antagonist L-753478 (10 mg/kg ip) on body weight change and food intake. The data for Exhibits 1 and 2 were analyzed for statistical significance with a one-way ANOVA coupled to a post-hoc Bonferonni/Dunn test was performed. P values < 0.05 were considered significant and were obtained as follows:

Study Protocol: For the studies male C57BL/6 mice (16 month old) were employed. The animals were housed individually in plastic cages kept at 23 ± 2°C, 55 ± 15% relative humidity, and maintained on a light-dark cycle with the lights on from 0700-1900 h. Water and chow were available *ad libitum*. This experiment used male Diet-Induced Obese (DIO) C57BL mice, which were fed moderately high fat (MHF) diet for about 10 months. On the test day, the NPY1 receptor antagonist L-753478 at 10 mpk [dissolved in ethanol/PEG400/saline (10:25:65)] or the

respective vehicle were intraperitoneally administered, and simultaneously, the NPY5 receptor antagonist L-753550 at 30 mpk or vehicle (0.5% methylcellulose) were orally administered. The administration was done one and half hours before the beginning of the dark period, and 24 hr food intake and body weight changes were measured.

Results: The NPY1 and NPY5 receptor antagonists were evaluated to see if they were either additive or synergistic with respect to feeding suppression and bodyweight gain. The orally administered NPY5 receptor antagonist L-753550 alone at 30 mg/kg tended to reduce food intake slightly by 9%, while intraperitoneally administered NPY1 receptor antagonist L-753478 alone at 10 mg/kg potently suppressed food intake by 27%. The combination of the NPY1 receptor antagonist L-753478 and NPY5 receptor antagonist L-753550 produced a greater suppression of food intake of 52%, and this combined effect was clearly greater than the theoretical summation (36%) of each single treatment. In addition, the combination treatment also produced greater reduction of body weight, then either single treatment. Single treatment of the NPY1 receptor antagonist L-753478 or NPY5 receptor antagonist L-753550 each produced a non-significant 0.3 gram (versus vehicle) weight loss, while the combination treatment produced a 0.9 gram weight loss, which was much more than additive, and which is consistent with being synergistic.

12. As can be seen from Exhibits 1 and 2, the combination of NPY5 receptor antagonist L-753550 and NPY1 receptor antagonist L-753478 produced an unexpectedly greater than additive, or synergistic, body weight loss and food intake reduction than either treatment alone.

Discussion of Food Intake and Body Weight Loss Results for Exhibits 1 and 2: As a practical matter we consider any combination that is 125% greater than the numerical sum of each treatment individually to be synergistic. Both the NPY1 receptor antagonist L-753478 and the NPY5 receptor antagonist L-753550 showed weak effects on food intake reduction and body weight loss. Surprisingly the effect on food intake reduction and body weight loss by the combination of NPY1 receptor antagonist L-753478 and NPY5 receptor antagonist L-753550 exceeded the sum of each single treatment result by at least 150%. Thus, the food intake and body weight data analyses indicated that the combination of L-753550 with L-753478 exerted a

synergistic effect on food intake reduction and body weight loss. The combination of L-753550 with L-753478 resulted in a greater food intake reduction and body weight loss than the hypothetical summation of each individual drug alone.

13. Thus, in my opinion, one of ordinary skill in the art would have found it surprising and unexpected that the combination of the NPY5 antagonist L-753550 and the NPY1 receptor antagonist L-753478 resulted in: 1) synergistic body weight loss, and 2) a synergistic reduction of food intake.

14. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Full Name: **Douglas John MacNeil**

Signature: Date:

Douglas John MacNeil Oct. 5, 2007

Exhibit 1

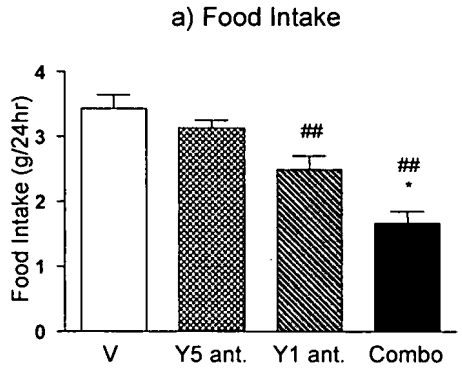
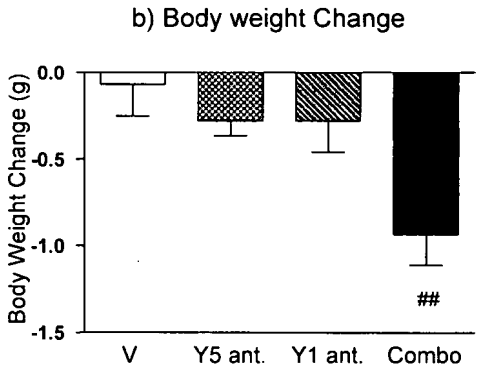


Exhibit 2



Exhibits 1 and 2: Combined anorexigenic effect of the Y1 and the Y5 antagonist in C57BL/6 DIO mice. V is vehicle; Y5 ant. is L-753550; Y1 ant. is J-115814 (also known as L-753478); and Combo is the combination of L-753550 and J-115814 (L-753478).

$p<0.01$ versus vehicle control group, * $p<0.05$ versus Y1 antagonist-treated group.

Exhibit 1. Synergistic effect of the combination of NPY5 antagonist (Y5 ant.) L-753550 and NPY1 antagonist (Y1 ant.) J-115814 (L-753478) on food intake reduction. The reduction in food intake over the 24 hour period was synergistic for the combination (Combo) of the NPY5 antagonist L-753,550 and the NPY1 receptor antagonist J-115814 (L-753478). Bars represent the amount of food in grams consumed by the DIO mice over 24 hours. The decrease in food intake for the combination is greater than what would be seen for an additive decrease in food intake.

Exhibit 2. Synergistic effect of the combination of NPY5 antagonist (Y5 ant.) L-753550 and NPY1 antagonist (Y1 ant.) J-115814 (L-753478) on body weight loss. The loss of body weight over the 24 hour period was synergistic for the combination (Combo) of the NPY5 antagonist L-753,550 and the NPY1 receptor antagonist J-115814 (L-753478). Bars represent the body weight change. The reduction in body weight for the combination is greater than what would be seen for an additive decrease in body weight.

Effects of Sibutramine Plus Orlistat in Obese Women Following 1 Year of Treatment by Sibutramine Alone: A Placebo-Controlled Trial

Thomas A. Wadden, Robert I. Berkowitz, Leslie G. Womble, David B. Sarwer, Marjorie E. Arnold, and Carrie M. Steinberg

Abstract

WADDEN, THOMAS A., ROBERT I. BERKOWITZ, LESLIE G. WOMBLE, DAVID B. SARWER, MARJORIE E. ARNOLD, AND CARRIE M. STEINBERG. Effects of sibutramine plus orlistat in obese women following 1 year of treatment by sibutramine alone: a placebo-controlled trial. *Obes Res.* 2000;8:431–437.

Objective: This study assessed whether adding orlistat to sibutramine would induce further weight loss in patients who previously had lost weight while taking sibutramine alone.

Research Methods and Procedures: Patients were 34 women with a mean age of 44.1 ± 10.4 years, weight of 89.4 ± 13.8 kg, and body mass index (BMI) of 33.9 ± 4.9 kg/m^2 who had lost an average of 11.6 ± 9.2% of initial weight during the prior 1 year of treatment by sibutramine combined with lifestyle modification. Patients were randomly assigned, in double-blind fashion, to sibutramine plus orlistat or sibutramine plus placebo. In addition to medication, participants were provided five brief lifestyle modification visits during the 16-week continuation trial.

Results: Mean body weight did not change significantly in either treatment condition during the 16 weeks. The addition of orlistat to sibutramine did not induce further weight loss as compared with treatment by sibutramine alone (mean changes = +0.1 ± 4.1 kg vs. +0.5 ± 2.1 kg, respectively).

Discussion: These results must be interpreted with caution because of the study's small sample size. The findings, however, suggest that the combination of sibutramine and orlistat is unlikely to have additive effects that will yield mean losses ≥15% of initial weight, as desired by many obese individuals.

Key words: sibutramine, orlistat, obesity, women, weight loss

Introduction

Two medications, sibutramine (Meridia; Knoll Pharmaceutical Co., Mt. Olive, NJ) (1) and orlistat (Xenical; Roche Laboratories, Nutley, NJ) (2), are currently approved by the Food and Drug Administration for weight loss and the maintenance of weight loss. Sibutramine is a combined norepinephrine-serotonin re-uptake inhibitor, whereas orlistat is a gastric and pancreatic lipase inhibitor. In controlled trials, sibutramine (15 mg once a day) was associated at 1 year with a 7% reduction in initial weight (1,3) and orlistat (120 mg, three times a day [TID]) with a 10% reduction (2,4–6). In both cases, the difference in weight loss between the medication and placebo conditions (i.e., placebo-subtracted weight loss) was approximately 4% to 5%.

Obese individuals want to lose two to three times more weight than is typically possible with current medications (7). Several investigators have suggested that larger weight losses might be achieved by combining weight loss agents (8–10). The present pilot study explored the benefits of adding orlistat to sibutramine in obese women who had lost an average of 11.6 ± 9.2% of their initial weight during 1 year of treatment by sibutramine alone. All women in the pilot study continued to receive sibutramine for 16 weeks; in addition, half of them were randomly assigned to orlistat and the other half to placebo. These two medications would appear to be excellent candidates for combined therapy because of their different mechanisms of action.

Submitted for publication April 3, 2000.
Accepted for publication in final form July 17, 2000.
University of Pennsylvania School of Medicine, Department of Psychiatry, Philadelphia, Pennsylvania.
Address correspondence to Thomas A. Wadden, Ph.D., University of Pennsylvania School of Medicine, 3600 Market Street, Suite 738, Philadelphia, PA 19104. E-mail: wadden@mail.med.upenn.edu

Research Methods and Procedures

Patients

Patients were 34 volunteers from a group of 43 women who had completed a 1-year treatment program that combined sibutramine (10 to 15 mg/d) with different amounts of lifestyle modification. As described in a separate report (11), the 43 participants lost an average of 12.0 ± 9.6 kg at 1 year, but there were marked differences among patients based on the program of lifestyle modification they received.

The 34 volunteers in the continuation study were told that all participants would receive sibutramine for an additional 16 weeks and that half of them also would be assigned at random (in double-blind fashion) to orlistat and the other half to placebo. The stated goal of the study was to determine whether the addition of orlistat would be associated with greater weight loss (or better maintenance of weight loss) than would continued treatment by sibutramine alone. Patients gave their informed consent to participate in the continuation study, which was approved by the University of Pennsylvania's Committee on Studies Involving Human Beings. Patients' characteristics, before randomization, are shown in Table 1. ANOVA showed that patients in the two conditions did not differ significantly on any of the baseline measures, including weight loss during the prior 1-year program.

Procedures

At baseline (i.e., week 52), all patients met with a physician (R. I. B.) who examined their health and told them to continue to take sibutramine (10 to 15 mg once a day) in the morning. In addition, they were instructed to take one capsule of the investigational medication within ± 1 hour of lunch, dinner, and an evening snack. We decided not to prescribe orlistat in the morning because 14 of 34 (41%) patients indicated that they ate breakfast infrequently (i.e., 0 to 3 times a week). Of the 20 remaining participants, 12 (i.e., 35% of the total sample) reported that they usually ate a breakfast that was determined to contain ≤10 g of fat. For most women, evening snacking appeared to present a greater risk for overeating than did breakfast.

Patients were instructed to limit their fat intake to a maximum of 20 g per meal (or snack), and 60 g per day, to minimize possible gastrointestinal events, including oily stools, oily spotting, fecal urgency, and related side effects (2,4–6). They were warned that they would not be able to predict the temporal occurrence of such events. Participants were instructed to take the medication three times a day, at the designated times, even if they missed a meal or snack. This was done to facilitate their taking the medication as regularly as possible. Patients were also instructed to take a multivitamin supplement every morning to prevent possible decreases in levels of fat-soluble vitamins.

At week 53, patients returned to see the physician who assessed their response to both sibutramine and the experimental medication. Follow-up medical visits were scheduled at weeks 56 and 68 (or more frequently, as needed).

Lifestyle Modification. All patients met with a registered dietitian or doctoral-level psychologist for 30 minutes at weeks 52, 56, 60, 64, and 68. At the first visit, patients' energy requirements were calculated, and they were instructed to consume a diet of 1200 to 1600 kcal/d, representing a deficit of approximately 600 to 850 kcal/d. Patients were told to consume a balanced diet (of their choosing) with approximately 20% of calories from protein, 50% from carbohydrate, and ≤30% from fat. They were provided handouts on topics that included the Food Guide Pyramid, food labels, low-fat cooking, and meal planning. Each month the practitioner reviewed patients' food diaries and medication compliance. Participants also set monthly activity goals with an eventual objective of exercising five times a week for 30 to 40 minutes per bout.

Dependent Measures. Weight was measured at each visit with patients dressed in light clothing and without shoes. At week 68 (i.e., end of study), participants indicated whether they believed they had been assigned to orlistat or placebo. In addition, they completed a symptom inventory that assessed, for the prior week, the number of days that they had experienced various gastrointestinal events.

Attrition and Statistical Analyses

Three patients treated by sibutramine plus orlistat (i.e., combined therapy) and five treated by sibutramine alone discontinued treatment prematurely. Table 2 summarizes the reasons for attrition and patients' weight loss at the time. A chi square test revealed no significant differences in dropout between conditions. Differences in weight loss between conditions during the 16-week trial were compared using analysis of covariance, with weight loss at the end of the first year of treatment (by sibutramine alone) taken as the covariate. Data were analyzed using both an end-point analysis (which included only treatment completers) and a last-observation-carried-forward analysis. The two sets of analyses reached the same statistical conclusions.

Results

Weight Loss

Figure 1 shows that body weight was essentially unchanged in both conditions during the 16-week continuation trial. ANOVA revealed neither an effect of time nor treatment condition. Thus, contrary to our hypothesis, the addition of orlistat to sibutramine did not significantly increase weight loss (or improve the maintenance of weight loss) as compared with the continued use of sibutramine alone (see Table 3).

A second ANOVA examined the effect of prior 1-year weight loss and treatment. Patients were divided into two groups based on whether they had lost <10% of their initial weight in the prior 1-year study or ≥10% (resulting in a 2 ×

Table 1. Patients' characteristics before randomization to sibutramine or sibutramine plus orlistat for the 16-week continuation study

Variable	Sibutramine plus placebo (N = 17)	Sibutramine plus orlistat (N = 17)
Age (years)	44.3 ± 10.4	43.9 ± 10.7
Weight (kg)	90.1 ± 14.4	88.7 ± 13.5
Height (cm)	163.6 ± 5.4	161.3 ± 10.1
BMI (kg/m^2)	33.6 ± 4.8	34.2 ± 5.1
Age of onset of obesity (year)	17.6 ± 10.5	14.1 ± 9.3
First year weight loss (kg) on sibutramine alone	9.8 ± 8.5	13.4 ± 9.7

There were no significant differences among groups on any of the above variables.

2 ANOVA). (The mean loss for the 16 patients in the first group was 3.3 ± 3.2%, whereas that for the 18 participants in the second group was 18.9 ± 5.8%.) Patients who had reduced <10% during the earlier trial lost 1.2 ± 3.2 kg during the 16-week continuation study, independent of which medications they received. By contrast, those who had lost >10% of weight in the prior trial gained 1.7 ± 2.6 kg during the 16-week study, yielding a significant difference between groups ($p < 0.01$). Figure 2 shows that women who had lost <10% of weight in the prior 1-year trial tended to lose more weight in the continuation study if assigned to orlistat plus sibutramine rather than to sibutramine alone (−2.6 ± 4.9 kg vs. −0.4 ± 1.2 kg, respectively). The difference, however, between conditions was not statistically significant.

A final subanalysis examined weight change in eight women who were assigned to the combination of orlistat plus sibutramine and had lost 5% to 14% of initial weight in the prior 1-year study. These women were selected, because all had responded to sibutramine (i.e., achieved a 5% weight loss) but had not lost so much weight (i.e., ≥15%) as to make further weight reduction unlikely with orlistat. These participants lost an average of 8.4 ± 4.4% of initial weight in the 1-year trial. In the 16-week continuation study, their mean weight increased by 0.2 ± 5.1 kg. Thus, even in highly selected patients, who were thought to be the most likely to benefit from combination therapy, adding orlistat to sibutramine did not increase weight loss.

Medication Dose

Of the 17 patients assigned to sibutramine plus placebo, 7 took 10 mg/d of sibutramine and 10 received 15 mg/d. In the sibutramine plus orlistat group, 6 took 10 mg/d of sibutramine while the other 11 participants received 15

Table 2. Summary of attrition for eight patients

Treatment condition	Reason for discontinuation*	Week	Weight change (in kg) at attrition
Sibutramine†	Lost to follow-up; dissatisfied with treatment	53	−1.0
Sibutramine	PCP removed due to BP: 157/88 mm Hg	56	+0.7
Sibutramine	Premature atrial contractions (found to be an unreported pre-existing condition)	64	+3.8
Sibutramine	Lost to follow-up; dissatisfied with treatment	56	−1.7
Sibutramine	Death in family	56	−1.8
Sibutramine + orlistat‡	Bronchitis and flu requiring hospitalization	64	+5.5
Sibutramine + orlistat	Lost to follow-up; dissatisfied with treatment	64	+0.8
Sibutramine + orlistat	Medical illness in family	53	−0.8

* PCP, primary care physician; BP, blood pressure.

† Sibutramine; mean number of weeks attended was 57 ± 4.1; mean weight change (in kg) at attrition was +0.0 ± 2.4.

‡ Sibutramine + orlistat: mean number of weeks attended was 60.3 ± 6.4; mean weight change (in kg) at attrition was +1.8 ± 3.3.

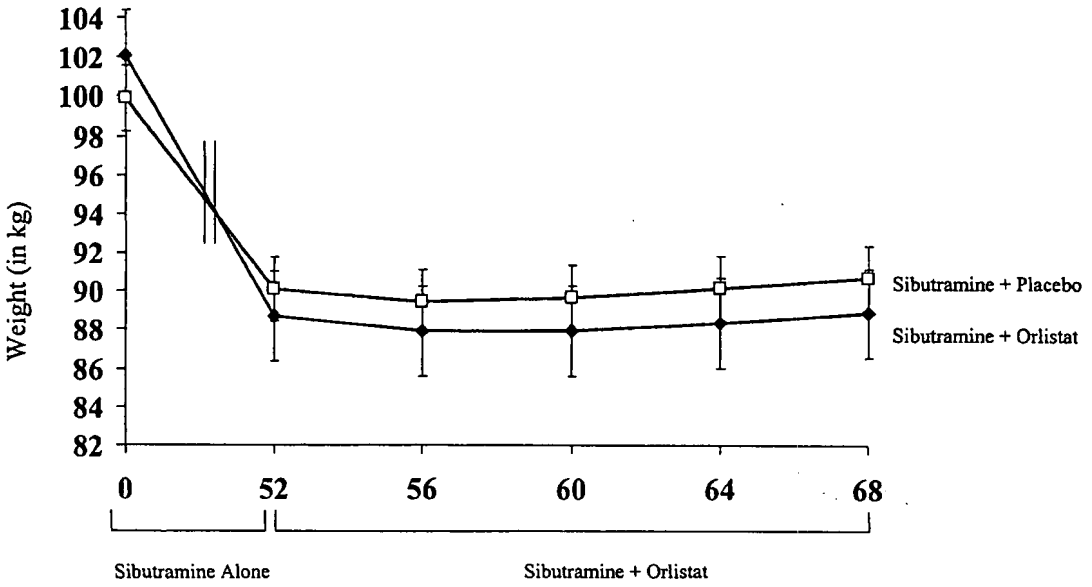


Figure 1. Change in body weight during the 16-week continuation trial for patients assigned to sibutramine plus placebo ($N = 17$) or sibutramine plus orlistat ($N = 17$).

mg/d. All patients had been prescribed the 15 mg/d dose in the original 1-year study but, by the end of the year, it had been reduced to 10 mg/d in 13 of 34 women to control side-effects that included insomnia and increased blood pressure and pulse. These reductions occurred before patients began the 16-week continuation trial. There were no significant differences in weight change during the 16-week trial between patients who received the 10 mg/d vs. 15 mg/d dose.

Determination of Treatment Condition

Of the 14 patients assigned to sibutramine plus orlistat, 12 correctly identified their treatment condition at the end of the trial, as did 10 of 12 assigned to sibutramine plus placebo. A chi square test revealed that the percentage of correct identifications (84.6%) was significantly ($p < 0.05$) greater than that expected by chance. Thus, patients appeared to know whether they had received orlistat.

Symptom Reports

Table 4 presents patients' reports of gastrointestinal symptoms during the last week of the trial. Fifty percent of patients treated by combined therapy reported experiencing soft stool and increased frequency of bowel movements at least 1 day of the week, as compared with only 9.1% of patients treated by sibutramine alone. Similarly, 42.9% of

Table 3. Change in weight (kg) for patients in two conditions

	Sibutramine plus placebo		Sibutramine plus orlistat	
Time	**EPA***	**LOCF†**	**EPA**	**LOCF**
Week 56	−0.7 ± 1.3	−0.7 ± 1.2	−0.9 ± 1.9	−0.7 ± 1.8
Week 60	−0.3 ± 1.6	−0.5 ± 1.4	−0.7 ± 3.1	−0.7 ± 2.9
Week 64	+0.2 ± 1.9	+0.1 ± 1.8	−0.6 ± 4.4	−0.4 ± 4.2
Week 68	+0.8 ± 2.0‡	+0.5 ± 2.1§	−0.3 ± 4.2¶	+0.1 ± 4.1‖

* EPA = end-point-analysis; N = 16, 10, 12, and 12 at weeks 56, 60, 64, and 68, respectively, for sibutramine plus placebo; N = 14, 15, 15, and 14 at weeks 56, 60, 64, and 68, respectively, for sibutramine plus orlistat.

† LOCF = last-observation-carried-forward analysis; $N = 17$ for both treatment conditions at all times.

Note: 95th percentile confidence intervals = ‡2.1 to −0.5, §1.6 to −0.5; ¶2.2 to −2.7, and ‖2.2 to −2.0.

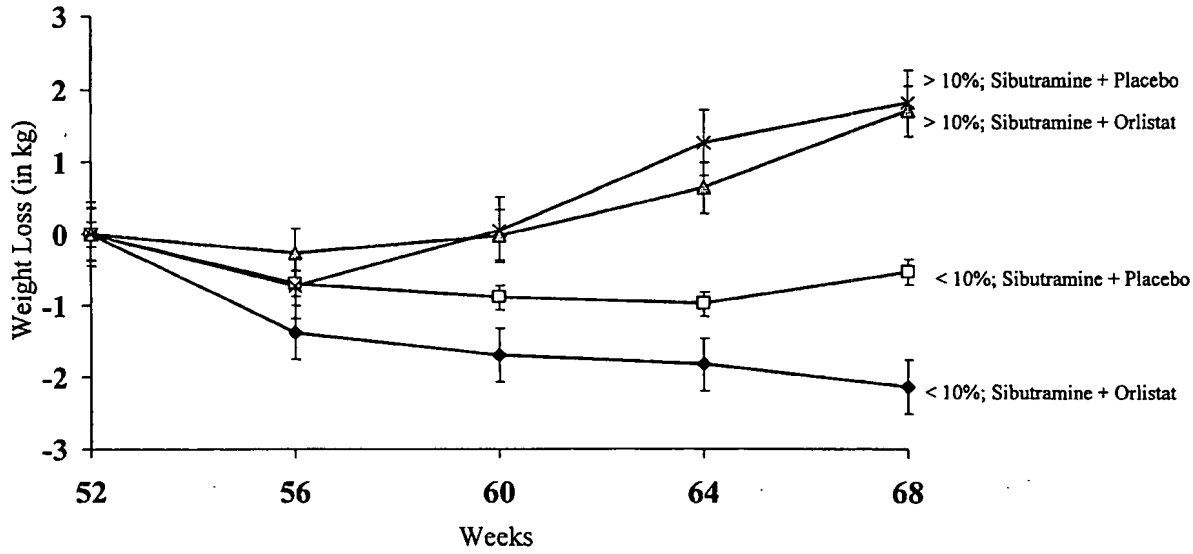


Figure 2. Change in body weight (from week 52) for patients who had lost <10% of initial weight in the prior 1-year trial and were assigned to sibutramine plus placebo (N = 9) or to sibutramine plus orlistat (N = 7). Data are also shown for patients who had lost >10% of initial weight in the prior trial and who received sibutramine plus placebo (N = 8) or sibutramine plus orlistat (N = 10).

combined-therapy patients reported oily evacuation and fecal urgency at least 1 day of the week as compared with 0% and 9.1%, respectively, of patients treated by sibutramine alone. Although three of these four differences were statistically significant at the 0.05 level, none was significant at the 0.004 level, the level required if Bonferroni's correction for multiple tests were used.

During physician visits, patients did not report any unusual symptoms that could not be attributed to either sibutramine or orlistat alone. Thus, combining the two medications did not appear to result in any unexpected side-effects.

Discussion

This study's principal finding was that adding orlistat to sibutramine did not significantly increase weight loss in obese women who had previously lost 11.6% of initial weight during 1 year of treatment by sibutramine alone. The two medications did not appear to have additive effects, a finding that disappointed several patients who had hoped, as we had, that they could lose approximately 10% of weight with the first medication and then an additional 10% with the second. The data revealed a trend for patients, who in the prior 1-year trial had lost less than 10% of their initial weight with sibutramine, to lose additional weight by also taking orlistat. However, their loss of only 2.6 kg at the end of 16 weeks was modest and did not differ significantly from that of patients who received sibutramine plus placebo. In addition, it is possible that these patients would have lost 2.6 kg if treated by orlistat alone (without combining it with sibutramine).

Patients who had lost ≥10% of initial weight in the prior 1-year trial appeared to receive little benefit from combined therapy; they gained 1.7 kg during the 16-week continuation study, as did patients treated by sibutramine alone. This finding suggests that there may be limits to the amount of weight that most obese individuals can lose (and maintain) with currently approved medications (8), as well as with behavioral interventions (12). This limit appears to be 10% to 15% of initial weight. Efforts to push beyond this limit may be thwarted by a toxic environment (13) that discourages physical activity while encouraging consumption of a high-fat diet, as well as by compensatory biological responses (14,15) that decrease energy expenditure. Whether alone or together, these factors appear to return weight toward the 10% mark, if not toward baseline (16–18). Andersen et al. (19), for example, used a very low calorie diet to induce an average loss of approximately 15% of initial weight but found that patients maintained a loss of only 10% at the end of 1 year, despite their receiving 30 mg/d dexfenfluramine throughout the trial. Hill et al. (20) similarly found during a 1-year follow-up that patients regained about one quarter of their 11% reduction in initial weight, despite receiving orlistat (120 mg TID) for the full follow-up period. From this perspective, it is not surprising that our most successful patients, who had lost an average of 18.9% in the prior 1-year trial, tended to gain weight in the 16-week continuation study, whether they received sibutramine alone or sibutramine plus orlistat. Even when a subanalysis was conducted on eight women who had lost a mean of only 8.6% of initial weight in the prior trial, they were

Table 4. Patients' report of side effects at week 68

	% of patients endorsing symptoms		
Symptom*	**Orlistat**	**Placebo**	***p* value**
Soft stool	50	9.1	0.04
Increased bowel movement	50	9.1	0.04
Fecal urgency	42.9	9.1	0.09
Oily evacuation	42.9	0	0.02
Oily spotting	28.6	9.1	NS*
Flatus with discharge	28.6	0	0.10
Fatty oily stool	28.6	0	0.08
Liquid stool	14.3	9.1	NS
Stomach pain upset stomach	14.3	9.1	NS
Fecal incontinence	7.1	0	NS
Decreased bowel movement	7.1	0	NS
Pellets/hard stool	7.1	18.2	NS

* NS, not significant.

found to gain 0.2 kg during the 16-week continuation study while receiving sibutramine plus orlistat.

Future medications, or combinations of medications, may well be capable of inducing and sustaining larger weight losses (8). This was the promise of the fenfluramine-phentermine combination until the fenfluramines were removed from the market in 1997 because of their association with valvular heart disease (21).

Results of the present study must be interpreted with caution because of our small sample size. Clearly, further studies are needed that have adequate power to detect clinically significant differences. In designing our investigation, we estimated that patients treated by orlistat plus sibutramine would lose 3.0 ± 3.0 kg during the 16-week trial, whereas those who received sibutramine alone would have a mean weight change of 0.0 ± 3.0 kg. With a sample size of 34, the power to detect this difference was 0.81 (α = 0.05, two-tailed test). We thought that, even with this small sample, we would be able to detect at least a trend toward significant differences between the two conditions.

In addition to increasing the sample size (and including men), investigators may wish to use alternative study designs such as comparing sibutramine (plus placebo) to orlistat (plus placebo) to the two medications combined (i.e., sibutramine plus orlistat). There are also a variety of options for sequencing the medications that include prescribing both from the outset or introducing the second medication only after the patient has met a weight-related criterion such as a 5% loss, a 2-month weight loss plateau, or significant weight regain. In addition, longer trials (≥1 year) will be needed to determine whether combined therapy improves the maintenance of weight loss. The present study was limited to 16 weeks, because we were interested primarily in whether adding orlistat to sibutramine would induce further weight loss. Maximum weight loss with medication typically occurs in the first 16 to 26 weeks (8).

We intentionally used a modest behavioral intervention in the continuation study to reveal most clearly the effects of the medications. Use of a more intensive lifestyle intervention may well have increased the size of the weight losses produced by combined therapy (as well as by sibutramine alone), as shown in a previous study (11).

The present findings raise questions about whether it is possible to conduct truly blinded evaluations of orlistat. At the end of the 16-week trial, all but 4 of 26 patients correctly identified their treatment condition. We suspect that the gastrointestinal side-effects associated with orlistat enabled patients to discern their treatment assignment. The problem, however, of patients becoming "unblinded" is not unique to orlistat. Nearly two decades ago Brownell and Stunkard (22) showed that 71% of patients correctly identified whether they had been assigned to fenfluramine or placebo.

In summary, results of this pilot study revealed little benefit of adding orlistat to sibutramine in patients who had previously lost 11.6% of initial weight on sibutramine. Additional studies, however, that include larger sample sizes, as well as different experimental designs, are needed to reach definitive conclusions about the possible benefits of combining these two medications.

Acknowledgments

The authors thank Dr. Richard Prus-Wisniewski for his contributions to this study, as well as Knoll Pharmaceutical Co. for providing the sibutramine used in the trial. Orlistat was purchased by the authors. This study was supported, in part, by a National Institute of Mental Health Research Scientist Development Award and by grants from Knoll Pharmaceutical Company and Novartis Nutrition Co. Drs. Berkowitz, Sarwer, and Wadden have all received speaking honoraria from Knoll Pharmaceutical Co. and Roche Laboratories, which manufacture sibutramine and orlistat, respectively. Drs. Berkowitz and Wadden also serve (or have served) as consultants to both companies.

References

1. **Knoll Pharmaceutical Co.** Meridia (sibutramine hydrochloride monohydrate). Prescribing information. In: *Physician's Desk Reference.* Montvale, NJ: Drug Information Services Group; 2000, pp. 1509–13.
2. **Roche Laboratories.** Xenical (orlistat). Prescribing information. In: *Physician's Desk Reference.* Montvale, NJ: Drug Information Services Group; 2000, pp. 2693–6.
3. **Lean MEJ.** Sibutramine: a review of clinical efficacy. *Int J Obes.* 1997;21:30S–6S.
4. **Davidson MH, Hauptman J, DiGirolamo M, et al.** Weight control and risk factor reduction in obese subjects treated for 2 years with orlistat: a randomized controlled trial. *JAMA.* 1999;281:235–42.
5. **Heymsfield SB, Segal KR, Hauptman J, et al.** Effects of weight loss with orlistat on glucose tolerance and progression to type 2 diabetes in obese adults. *Arch Int Med.* 2000;160:1321–6.
6. **Sjöström L, Rissanen A, Anderson T, et al.** Randomized placebo-controlled trial of orlistat for weight loss and prevention of weight regain in obese patients. *Lancet.* 1998;352:167–73.
7. **Foster GD, Wadden TA, Vogt RA.** What is a reasonable weight loss?: patients' expectations and evaluations of obesity treatment outcomes. *J Consult Clin Psychol.* 1997;65:79–85.
8. **Bray GA, Greenway FL.** Current and potential drugs for treatment of obesity. *Endocr Rev.* 2000;20:805–75.
9. **Atkinson RL, Blank RC, Schumacher D, Dhurandhar NV, Ritch DL.** Long-term drug treatment of obesity in a private practice setting. *Obes Res.* 1997;5:578–86.
10. **Weintraub M, Sundaresan PR, Madan M, et al.** Long-term weight control study I (weeks 0 to 34): the enhancement of behavior modification, caloric restriction, and exercise by fenfluramine plus phentermine versus placebo. *Clin Pharmacol Ther.* 1992;51:586–94.
11. **Wadden TA, Berkowitz RI, Sarwer DB, Prus-Wisniewski R, Steinberg CM.** Benefits of lifestyle modification in the pharmacologic treatment of obesity: a randomized trial. *Arch Int Med.*, in press.
12. **Wadden TA, Sarwer DB, Berkowitz RI.** Behavioral treatment of the overweight patient. *Bailliere's Clin Endocrin Metab.* 1999;13:93–107.
13. **Brownell KD.** *The Learn Program for Weight Management 2000.* Dallas, TX: American Health Publishing Co. 2000, pp. 209–10.
14. **Ravussin E, Swinburn BA.** Effect of caloric restriction and weight loss on energy expenditure. In: Wadden TA, VanItallie TB, ed. *Treatment of the Seriously Obese Patient.* New York: Guilford Press; 1992, pp. 163–89.
15. **Campfield LA, Smith FJ, Burn P.** The OB protein (leptin) pathway: a link between adipose tissue mass and central neural networks. *J Metab Res.* 1996;28:619–32.
16. **Stunkard AJ.** Mini review: anorectic agents lower a body weight set point. *Life Sci.* 1982;30:2043–55.
17. **Andersen T, Astrup A, Quaade F.** Dexfenfluramine as adjuvant to a low-calorie formula diet in the treatment of obesity: a randomized clinical trial. *Int J Obes.* 1992;16:35–40.
18. **Wadden TA, Foster GD, Letizia KA.** One-year behavioral treatment of obesity: comparison of moderate and severe caloric restriction and the effects of maintenance therapy. *J Consult Clin Psychol.* 1994;62:165–71.
19. **Andersen T, Astrup A, Quaade F.** Dexfenfluramine as adjuvant to a low-calorie formula diet in the treatment of obesity: a randomized clinical trial. *Int J Obes.* 1992;16:35–40.
20. **Hill JO, Hauptman J, Anderson JW, et al.** Orlistat, a lipase inhibitor, for weight maintenance after conventional dieting: a 1-year study. *Am J Clin Nutr.* 1999;69:1108–16.
21. **Connolly HM, Crary JL, McGoon MD, et al.** Valvular heart disease associated with fenfluramine-phentermine. *N Engl J Med.* 1997;337:581–8.
22. **Brownell KD, Stunkard AJ.** The double-blind in danger: untoward consequences of informed consent. *Am J Psychiatry.* 1982;139:1487–9.

Presented at NAASO, Obesity Society annual meeting
Boston MA
October 2006

#2114 #54

NPY5R Antagonism Does Not Augment the Weight Loss Efficacy of Orlistat or Sibutramine

Ngozi Erondu,[1] Carol Addy,[1] Ira Gantz,[1] Bret Musser,[1] Kaifeng Lu,[1] Madhuja Mallick,[1] Joseph Proietto,[2] Arne Astrup,[3] Søren Toubro,[3] Aila M. Rissannen,[4] Serena Tonstadt,[5] William G. Haynes,[6] Keith M. Gottesdiener,[1] Keith D. Kaufman,[1] John Amatruda,[1] Steven B. Heymsfield[1]

[1]Merck Research Laboratories, Rahway, NJ and Boston, MA; [2]Royal Melbourne Hospital, Dept. of Medicine, Melbourne, Australia; [3]KVL, Forsk. Inst. f. Human Ernæring, Frederiksberg, Danmark; [4]Helsinki University Central Hospital, Obesity Research Unit, Helsinki, Finland; [5]Ulleval Universitetssykehus HF Klinikk for forebyggende medisin, OSLO, Norway; [6]University of Iowa, Iowa City, IA

MERCK

Abstract

[illegible]

Introduction

[illegible]

Methods

[illegible]

Results

[illegible]

Summary

[illegible]

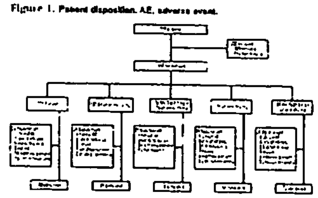


Figure 1. Patient disposition. AE, adverse event.

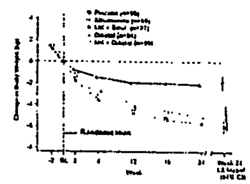


Figure 2. Mean change from baseline in body weight (kg) over 24 weeks of treatment using last observation carried forward (Modified Intention to Treat Population)
[1]Least Squares (LS) Mean estimates (84% Confidence interval) based on ANCOVA model with terms for treatment, baseline body weight, center, and run-in weight change.

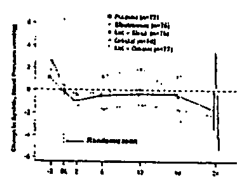


Figure 3. Change from baseline in systolic blood pressure (mmHg) over 24 weeks of treatment (Modified Intention to Treat Population). Observed data plotted from Week -2 to 24. Sample size corresponds to Week 24. 84% Confidence interval shown on Week 24.

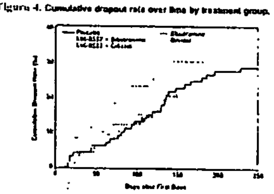


Figure 4. Cumulative dropout rate over time by treatment group.

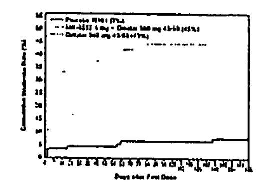


Figure 5. Time to first event per patient, expressed as cumulative incidence rate, of gastrointestinal adverse experiences by treatment group for the Modified Intention to Treat Population.

Table 1. Patient baseline characteristics for all randomized patients.

	Placebo	Sibutramine	MK-0557 + Sibutramine	Orlistat	MK-0557 + Orlistat
Race (white)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Gender (women)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Age (years)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
BMI (kg/m²)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Weight (kg)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

Table 2. Selected metabolic and CV outcomes for MITT population.

	Baseline	Week 24	LS change
Weight (kg)			
Placebo	[illegible]	[illegible]	[illegible]
Sibutramine	[illegible]	[illegible]	[illegible]
MK-0557 + Sibutramine	[illegible]	[illegible]	[illegible]
Orlistat	[illegible]	[illegible]	[illegible]
MK-0557 + Orlistat	[illegible]	[illegible]	[illegible]
Systolic blood pressure (mmHg)			
Placebo	[illegible]	[illegible]	[illegible]
Sibutramine	[illegible]	[illegible]	[illegible]
MK-0557 + Sibutramine	[illegible]	[illegible]	[illegible]
Orlistat	[illegible]	[illegible]	[illegible]
MK-0557 + Orlistat	[illegible]	[illegible]	[illegible]
HDL Cholesterol (mg/dL)			
Placebo	[illegible]	[illegible]	[illegible]
Sibutramine	[illegible]	[illegible]	[illegible]
MK-0557 + Sibutramine	[illegible]	[illegible]	[illegible]
Orlistat	[illegible]	[illegible]	[illegible]
MK-0557 + Orlistat	[illegible]	[illegible]	[illegible]
Triglycerides (mg/dL)			
Placebo	[illegible]	[illegible]	[illegible]
Sibutramine	[illegible]	[illegible]	[illegible]
MK-0557 + Sibutramine	[illegible]	[illegible]	[illegible]
Orlistat	[illegible]	[illegible]	[illegible]
MK-0557 + Orlistat	[illegible]	[illegible]	[illegible]
LDL Cholesterol (mg/dL)			
Placebo	[illegible]	[illegible]	[illegible]
Sibutramine	[illegible]	[illegible]	[illegible]
MK-0557 + Sibutramine	[illegible]	[illegible]	[illegible]
Orlistat	[illegible]	[illegible]	[illegible]
MK-0557 + Orlistat	[illegible]	[illegible]	[illegible]

Data at Baseline and Week 24 are observed mean (SD) except for triglycerides (TG) that are median (SD). Data for change from baseline are LS mean change (95% CI) except for triglycerides (TG) that are median change (95% CI).

Table 3. Clinical Adverse Experience Summary.

Number (%) of patients:	Placebo n	(%)	Sibutramine 10 mg n	(%)	MK-0557 1 mg + Sibutramine 10 mg n	(%)	Orlistat 360 mg n	(%)	MK-0557 1 mg + Orlistat 360 mg n	(%)
With one or more adverse experiences	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
With no adverse experience	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
With drug-related adverse experiences[1]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
With serious adverse experiences	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
With serious drug-related adverse experiences	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Who died	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Discontinued due to adverse experiences	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Discontinued due to drug-related adverse experiences	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Discontinued due to serious adverse experiences	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Discontinued due to serious drug-related adverse experiences	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[1]Determined by the investigator to be possibly, probably or definitely drug related.

References

[illegible]

F3143RY01.al 10/12/06 Job#1189 Heymsfield
Final size: 7'6" w x 3'8" h
NAASO

NPY5R Antagonism Does Not Augmen

Ngozi Erondu,[1] Carol Addy,[1] Ira Gantz,[1] Bret Musser,[1] Kaifeng Lu,[1] Madhuja Mallic
Keith M. Gottesdiener,[1] Keit
[1]Merck Research Laboratories, Rahway, NJ and Boston, MA; [2]Royal Melbourne Hospital, Dept. of Medicine, Melbou
Finland; [5]Ulleval Universitetssykehus HF, Klin

Abstract

Background: Currently approved drugs for weight control, sibutramine and orlistat, have limited efficacy, which may be related to counter-regulatory mechanisms including the orexigenic neuropeptide Y (NPY) pathway. The objective of this study was to evaluate whether MK-0557, a highly selective NPY Y5 receptor (NPY5R) antagonist, potentiates the weight loss effects of sibutramine and orlistat.

Research Methods and Procedures: We conducted a double-blind, placebo-controlled study which randomized 497 obese patients (BMI 30-43 kg/m^2) to 1 of 5 treatment arms [placebo (n=101); sibutramine 10 mg q.d. (n=100); MK-0557 1 mg q.d. plus sibutramine 10 mg q.d. (n=98); orlistat 120 mg t.i.d. (n=99); MK-0557 1 mg q.d. plus orlistat 120 mg t.i.d. (n=99)] in conjunction with a hypocaloric diet and exercise.

Results: In the Modified Intention to Treat population, imputing missing data using Last Observation Carried Forward, the least squares (LS) mean difference (95% CI) between MK-0557 + sibutramine and sibutramine alone was 0.1 (-1.6, 1.4) kg (p=0.892) and between MK-0557 + orlistat and orlistat alone was 0.9 (-2.4, 0.6) kg (p=0.250). Sibutramine alone induced an LS mean weight loss of -5.9 (-6.8, -4.9) kg versus -4.6 (-5.7, -3.6) kg for orlistat. Seventy-one percent in the placebo, 76% in the sibutramine alone, 80% in the MK-0557 + sibutramine, 69% in the orlistat alone, and 76% in the MK-0557 + orlistat groups completed the study.

Conclusions: In this study, blockade of the NPY5R with MK-0557 did not increase the weight loss efficacy of either orlistat or sibutramine. Sibutramine was associated with greater weight loss and better patient retention than orlistat, although the differences between the two drugs were not statistically significant.

Introduction

Two presently approved medications for weight loss treatment are orlistat and sibutramine. Orlistat is an inhibitor of gastrointestinal and pancreatic lipases that promotes weight loss and negative energy balance through reducing fat absorption (1, 2). Sibutramine is a selective inhibitor of the reuptake of norepinephrine and serotonin and, to a lesser extent, dopamine, which facilitates weight loss through both suppression of food intake and augmentation of energy expenditure (3, 4).

While the orlistat and sibutramine development programs were initiated over two decades ago, new novel targets are beginning to be critically evaluated with translational research. In this respect, neuropeptide Y (NPY) has been characterized as a potent orexigenic factor that is a key component of an anabolic network that promotes food intake and decreases energy expenditure (5-9). MK-0557 is a highly selective NPY5R antagonist that induced modest, dose-dependent weight loss in a 12-week proof-of-concept clinical trial in obese patients (10). This dose-ranging study combined with receptor occupancy data from positron emission tomography (PET, 10) established 1 mg as the appropriate daily dose of MK-0557 for clinical studies.

As part of our evaluation of MK-0557 as a clinical candidate we examined the weight-loss effects of this NPY5R antagonist when co-administered with orlistat and sibutramine. Our experimental protocol also provided the opportunity for a head-to-head comparison of orlistat and sibutramine.

Methods

Hypotheses and Study Design

Primary hypotheses: MK-0557 1 mg q.d. co-administered with (1) sibutramine 10 mg q.d. for 24 weeks reduces body weight more than sibutramine alone; (2) orlistat 120 mg t.i.d. for 24 weeks reduces body weight more than orlistat alone; and (3) sibutramine or orlistat for 24 weeks is safe and well tolerated.

The hypothesis was examined in a multicenter, double blind, randomized, placebo-controlled study. Prior to randomization, there was a 2-week diet/exercise and single blind placebo run-in period. Patients were instructed to follow a diet 500 kcal/day below their weight maintenance requirements, based upon an estimation of energy expenditure (11).

Eligible patients were randomized equally (Figure 1) to each of 5 treatment arms (placebo; sibutramine 10 mg q.d.; MK-0557 1 mg q.d. plus sibutramine 10 mg q.d.; orlistat 120 mg t.i.d.; MK-0557 1 mg q.d. plus orlistat 120 mg t.i.d.) and continued diet/exercise counseling. The primary measure of efficacy was change from baseline in body weight.

Obese patients with BMI between 30 kg/m^2 and 43 kg/m^2, between the ages of 18 and 65 years, inclusive, and who met other entry criteria were eligible to participate.

Statistical Analysis

The primary analysis population was a modified intention to treat (MITT) population, which was composed of subjects who received at least one dose of randomized study medication and had at least one post-randomization/baseline weight measurement. For evaluation of change from baseline, patients who had both a baseline and at least one post-baseline measurement were included in the analysis. Missing data were imputed using the last observation carried forward (LOCF). A repeated measures ANCOVA was also used to analyze the observed (i.e., without LOCF imputation); results were consistent with the LOCF analysis and are thus not reported here.

The efficacy hypotheses were evaluated by comparing the mean change from baseline in body weight using an analysis of covariance (ANCOVA) model with terms for weight loss during the run-in, baseline body weight, treatment, and center.

This study was powered to detect a 2.3 (2.0) kg difference with 90% (80%) power, assuming a standard deviation of 4.76 kg, level 0.05 for the primary hypotheses and 90 patients per treatment arm. The expected half-width of the 95% confidence interval was 1.4 kg.

Results

Patients

Patient characteristics are summarized in Table 1. Overall, the study consisted mainly of white (~75-83%) women (~80-85%) who were moderately obese with a baseline BMI of ~35 kg/m^2.

Patient disposition is outlined in Figure 1. A total of 719 patients were screened and from these 497 patients were randomized to placebo (n=101), sibutramine (n=100), MK-0557 + sibutramine (n=98), orlistat (n=99), and MK-0557 + orlistat (n=99). At completion of the protocol, 73% (n=368) of the 497 patients remained in the study, 71% (n=72) in the placebo group, 76% (n=76) in the sibutramine group, 79% (n=77) in the MK-0557 + sibutramine group, 69% (n=68) in the orlistat group, and 76% (n=75) in the MK-0557 + orlistat group.

MK-0557 + sibutramine versus sibutramine alone

After 24 weeks of treatment, MK-0557 did not induce significant weight loss when co-administered with sibutramine compared to sibutramine alone (p=0.892) (Table 2 and Figure 2). In the MITT population, the least squares (LS) mean difference (95% CI) between MK-0557 + sibutramine and sibutramine alone was -0.1 (-1.6, 1.4) kg. No significant differences were observed in the per-protocol population or in the 5% and 10% responder analyses.

MK-0557 + orlistat versus orlistat

After 24 weeks of treatment, MK-0557 did not induce significant weight loss when coadministered with orlistat compared to orlistat alone (p=0.250) (Table 2 and Figure 2). In the MITT population, the LS mean difference (95% CI) between MK-0557 + orlistat and orlistat alone was -0.9 (-2.4, 0.6) kg. No significant differences were observed in the per-protocol population or in the 5% and 10% responder analyses.

Sibutramine versus orlistat

The least squares (LS) mean change in body weight (95% CI) was -5.9 (-6.9, -4.9) kg in the sibutramine group and -4.6 (-5.7, -3.6) kg in the orlistat group, as compared to a mean change of -1.8 (-2.9, -0.8) kg for placebo (Figure 2 and Table 2). Both sibutramine and orlistat induced statistically significant changes in body weight (p<0.001 for both compounds vs. placebo), and the difference between the two compounds approached significance (p=0.097). No significant differences between sibutramine and orlistat were observed in the per-protocol population or in the 5% and 10% responder analyses.

Clinical evaluations and adverse events

Systolic (and diastolic) blood pressure was almost unchanged over 24 weeks in the placebo group (Table 2). Orlistat treatment was accompanied by a small reduction in both systolic and diastolic blood pressure (-1.4 and -1.2 mmHg, respectively), while sibutramine treatment was accompanied by a 2.1 mmHg elevation in systolic blood pressure. The systolic blood pressure difference for orlistat and sibutramine (3.5 mmHg; 95% CI: 0.9, 6.1 mmHg) was significant (p=0.008) while no significant treatment difference was observed in diastolic blood pressure. The temporal relations in systolic blood pressure changes over the study period are shown from the per-protocol population in Figure 3.

There were no deaths or serious drug-related adverse events (Table 3). The highest proportions of patients with drug-related adverse experiences were in the orlistat groups (40.4% for orlistat alone and 51.5% for orlistat + MK-0557) followed by the sibutramine (28.0% for sibutramine alone and 31.6% for sibutramine + MK-0557) and placebo (17.8%) groups. Of the patients discontinuing due to a drug-related adverse experience, 4% were in the orlistat alone and 1% in both the sibutramine alone and placebo groups. The study dropout rate (Figure 4) was highest in the orlistat alone group (31%) and lowest in the Sibutramine + MK-0557group (21%).

The most common reported clinical adverse experiences for the sibutramine group were dry mouth (6% vs. 1% in placebo and 1% in orlistat groups) and constipation (11% vs. 4% in placebo and 2% in orlistat groups). Diarrhea (17.2% vs. 3% in placebo and 5% in sibutramine groups), loose stools (8.1% vs. 2% in placebo and 1% in sibutramine groups), and other related gastrointestinal effects were the most common adverse events in the orlistat group. The orlistat-related gastrointestinal events tended to occur within the first four-weeks of treatment (Figure 5).

Summary

Our main observation is that in a randomized controlled clinical trial NPY5R antagonism with MK-0557 did not lead to additional weight loss beyond that observed with either sibutramine or orlistat alone. The mechanism(s) leading to lack of additivity are unknown but include statistical and biological hypotheses.

The current investigation is one of the first randomized, double-blind studies that include a head-to-head comparison of orlistat and sibutramine. In the present 24-week study, orlistat alone or in combination with MK-0557 led to significant weight loss compared to placebo of 2.8 and 3.7 kg, respectively. The respective weight loss above placebo at 24 weeks observed in our study for sibutramine with or without MK-0557, 4.2 kg and 4.0 kg, was greater than that with orlistat, although the differences were not statistically significant.

Our findings and those other head-to-head comparison studies suggest the overall weight loss efficacy of the two drugs is similar, with a small numerical advantage for sibutramine. However, the two drugs were not equally well tolerated. In our study, dry mouth and constipation were the two most frequently reported adverse events with sibutramine and retention of patients in the two sibutramine groups was similar to that of the placebo group. In contrast, gastrointestinal adverse events were pervasive and most likely accounted for the relatively high early dropout rate in both orlistat groups. Blood pressure, as expected, declined modestly (~1-2 mmHg) with orlistat treatment and increased to about the same extent in the sibutramine-treated patients.

In summary, our study demonstrated that the co-administration of a selective NPY5R antagonist with either of two conventional weight loss therapies, orlistat or sibutramine, did not result in a statistically significant

ent the Weight Loss E

allick,[1] Joseph Proietto,[2] Arne Astrup,[3] Sø
Keith D. Kaufman,[1] John Amatruda,[1] Steve
elbourne, Australia; [3]KVL, Forsk. Inst. f. Human Ern
F Klinikk for forebyggende medisin, OSLO, Norway; [6]

Figure 1. Patient disposition. AE, adverse event.

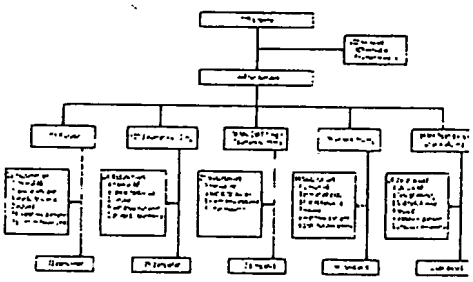


Figure 2. Mean change from baseline in body weight (kg) over 24 weeks of treatment using last observation carried forward (Modified Intention to Treat Population)
[1]Least Squares (LS) Mean estimates (84% Confidence Interval) based on ANCOVA model with terms for treatment, baseline body weight, center, and run-in weight change.

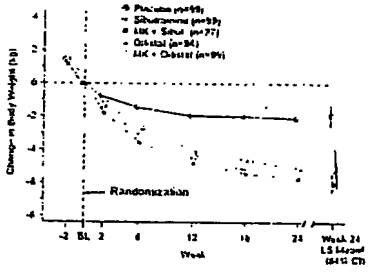
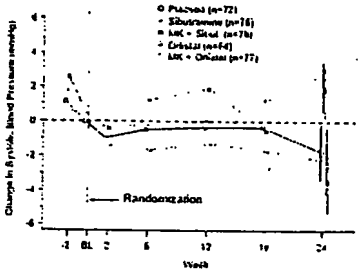


Figure 3. Change from baseline in systolic blood pressure (mmHg) over 24 weeks of treatment (Modified Intention to Treat Population). Observed data plotted from Week -2 to 24. Sample size corresponds to Week 24. 84% Confidence Interval shown on Week 24.



Presented at NAA-O, obesity society annual meeting Boston MA October 2006

#21141 #54

icacy of Orlistat or Sibutramine

Toubro,[3] Aila M. Rissannen,[4] Serena Tonstadt,[5] William G. Haynes,[6]
B. Heymsfield[1]
g, Frederiksberg, Denmark; [4]Helsinki University Central Hospital, Obesity Research Unit, Helsinki,
ersity of Iowa, Iowa City, IA

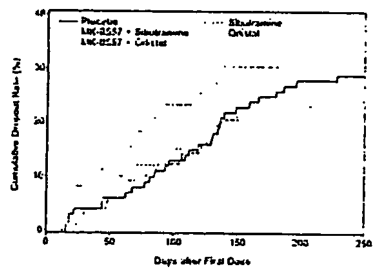


Figure 4. Cumulative dropout rate over time by treatment group.

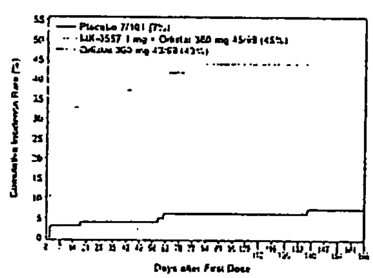


Figure 5. Time to first event per patient, expressed as cumulative incidence rate, of gastrointestinal adverse experiences by treatment group for the Modified Intention to Treat Population.

Table 1. Patient baseline characteristics for all randomized patients.

	Placebo (n=101)	Sibutramine (n=100)	MK-0557 + Sibutramine (n=98)	Orlistat (n=99)	MK-0557 + Orlistat (n=99)
Race (white)*	84 (83.2%)	77 (77.0%)	79 (80.6%)	78 (78.8%)	75 (75.8%)
Gender (women)*	83 (82.2%)	85 (85.0%)	79 (80.6%)	83 (83.8%)	79 (79.8%)
Age (years)[†]	42.6 (10.8)	40.9 (11.1)	40.7 (9.3)	41.9 (9.4)	42.4 (10.5)
BMI (kg/m²)[†]	35.9 (3.5)	35.8 (3.8)	35.3 (3.4)	35.3 (3.6)	35.7 (3.6)
Weight (kg)[†]	97.3 (15.2)	98.0 (15.4)	95.1 (13.5)	56.3 (12.3)	97.1 (14.0)

*Data are number (%). [†]Data are mean (SD)

Table 2. Selected metabolic and CV outcomes for MITT population.

	Baseline	Week 24	LS change
Weight (kg)			
Placebo	97.3 (15.2)	95.2 (16.1)	-1.8 (-2.9, -0.8)
Sibutramine	98.0 (15.4)	92.2 (15.8)	-5.9 (-6.9, -4.9)
MK-0557 + Sibutramine	96.1 (13.5)	90.4 (14.7)	-6.0 (-7.1, -4.9)
Orlistat	96.3 (12.3)	91.2 (12.7)	-4.6 (-5.7 -3.6)
MK-0557 + Orlistat	97.1 (14.0)	91.5 (14.8)	-5.5 (-6.6 -4.5)
Systolic blood pressure (mmHg)			
Placebo	117.7 (11.2)	117.8 (12.9)	0.1 (-1.7, 1.9)
Sibutramine	117.3 (11.0)	119.6 (10.9)	2.1 (0.3, 3.8)
MK-0557 + Sibutramine	115.4 (11.5)	117.5 (12.2)	1.2 (-0.6, 3.1)
Orlistat	116.6 (10.7)	115.5 (11.8)	-1.4 (-3.2, 0.5)
MK-0557 + Orlistat	118.1 (11.0)	115.1 (11.4)	-2.8 (-4.7, -1.0)
HDL Cholesterol (mg/dL)			
Placebo	49.3 (9.2)	49.5 (10.0)	1.0 (-1.7, 3.8)
Sibutramine	50.1 (12.9)	51.3 (12.0)	3.5 (0.8, 6.2)
MK-0557 + Sibutramine	49.2 (12.8)	51.0 (12.9)	6.2 (3.4, 8.9)
Orlistat	49.5 (11.9)	49.3 (10.9)	0.5 (-2.3, 3.4)
MK-0557 + Orlistat	49.9 (11.4)	49.3 (12.7)	1.1 (-1.7, 3.9)
Triglycerides (mg/dL)			
Placebo	109.5 (53.0)	119.0 (69.8)	3.3 (-5.8, 12.4)
Sibutramine	115.5 (71.5)	106.5 (70.7)	-9.0 (-16.1, -1.8)
MK-0557 + Sibutramine	119.0 (87.4)	101.0 (68.8)	-6.0 (-14.7, 2.7)
Orlistat	129.0 (75.3)	126.0 (56.6)	-2.2 (-9.6, 5.3)
MK-0557 + Orlistat	112.0 (47.4)	111.0 (78.1)	1.5 (-9.3, 12.3)
LDL Cholesterol (mg/dL)			
Placebo	111.8 (30.9)	114.0 (30.6)	7.8 (3.9, 11.6)
Sibutramine	115.4 (27.7)	120.2 (28.9)	5.5 (1.7, 9.3)
MK-0557 + Sibutramine	114.1 (25.7)	117.6 (32.6)	2.5 (-1.4, 6.3)
Orlistat	114.5 (27.8)	114.0 (29.1)	0.5 (-3.5, 4.5)
MK-0557 + Orlistat	113.8 (29.0)	107.4 (31.5)	-4.6 (-8.5, -0.7)

Data at Baseline and Week 24 are observed mean (SD) except for triglycerides (TG) that are median (SD). Data for change from baseline are LS mean change (95% CI) except for triglycerides (TG) that are median change (95% CI).

Table 3. Clinical Adverse Experience Summary.

	Placebo (N = 101)		Sibutramine 10 mg (N = 100)		MK-0557 1 mg + Sibutramine 10 mg (N = 98)		Orlistat 360 mg (N = 99)		MK-0557 1 mg + Orlistat 360 mg (N = 99)	
Number (%) of patients:	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
With one or more adverse experiences	68	(67.3)	68	(68.0)	64	(65.3)	69	(69.7)	84	(84.8)
With no adverse experience	33	(32.7)	32	(32.0)	34	(34.7)	30	(30.3)	15	(15.2)
With drug-related adverse experiences[1]	18	(17.8)	28	(28.0)	31	(31.6)	40	(40.4)	51	(51.5)
With serious adverse experiences	5	(5.0)	8	(8.0)	2	(2.0)	2	(2.0)	2	(2.0)
With serious drug-related adverse experiences	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Who died	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Discontinued due to adverse experiences	1	(1.0)	4	(4.0)	6	(6.1)	6	(6.1)	7	(7.1)
Discontinued due to drug-related adverse experiences	1	(1.0)	1	(1.0)	3	(3.1)	4	(4.0)	5	(5.1)
Discontinued due to serious adverse experiences	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Discontinued due to serious drug-related adverse experiences	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)

[1]Determined by the investigator to be possibly, probably or definitely drug related

References

1. Hagan S, Fenny A, Hadvary P, Lengsfeld H, Meier MK, Triscari J, Sullivan AC. Studies on the mechanism of action of tetrahydrolipstatin, a potent and selective inhibitor of pancreatic lipase. Int J Obes. 1987;11 Suppl 3:35-42.
2. Heck AM, Yanovski JA, Calis KA. Orlistat, a new lipase inhibitor for the management of obesity. Pharmacotherapy. 2000 Mar;20(3):270-9.
3. Poston WS, Foreyt JP. Sibutramine and the management of obesity. Expert Opin Pharmacother. 2004 Mar;5(3):633-42.
4. Halford JC, Harrold JA, Lawton CL, Blundell JE. Serotonin (5-HT) drugs: effects on appetite expression and use for the treatment of obesity. Curr Drug Targets. 2005 Mar;6(2):201-13.
5. Clark JT, Kalra PS, Crowley WR, Kalra SP. Neuropeptide Y and human pancreatic polypeptide stimulate feeding behavior in rats. Endocrinology. 1984 Jul;115(1):427-9.
6. Kalra SP, Dube MG, Pu S, Xu B, Horvath TL, Kalra PS. Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. Endocr Rev. 1999 Feb;20(1):68-100.
7. Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG. Central nervous system control of food intake. Nature. 2000 Apr 6;404(6778):661-71.
8. Sawchenko PE. Toward a new neurobiology of energy balance, appetite, and obesity: the anatomists weigh in. J Comp Neurol. 1998 Dec 28;402(4):435-41.
9. Elmquist JK, Elias CF, Saper CB. From lesions to leptin: hypothalamic control of food intake and body weight. Neuron. 1999 Feb;22(2):221-32.
10. Erondu N, Gantz I, Musser B, et al. Neuropeptide Y5 receptor antagonism does not induce clinically meaningful weight loss in overweight and obese adults. Cell Metab. 2006 Oct;4(4):275-82.
11. James WP, Avenell A, Broom J, Whitehead J. A one-year trial to assess the value of orlistat in the management of obesity. Int J Obes Relat Metab Disord. 1997;21 Suppl 3:S24-30.
12. Erondu, N, Wadden T, Gantz I, Musser B, Martin-Nguyen A, Bays H, Bray G, O'Neil P, Basdevant A, Amatruda JM, Kaufman KD, Heymsfield SB. Effect of NPY5R antagonist MK-0557 on weight regain after VLCD-induced weight loss (submitted).

F3143RY01.ai 10/12/06 job#1189 Heymsfield